

AQUA Cloning

1. Mix DNA fragments in a total volume of 10 µl dH2O with molar ratios of 3:1 (insert:vector) and 12 ng of linearized vector per 1 kb vector size
2. Incubate DNA-mixture at room temperature for 1 h
3. Transform in 25 (**50**) µl aliquot of competent E.coli cells
 1. Thawed on ice, 5 (**10**) µl DNA-mixture added
 2. Incubation for 10 (**30**) min on ice
 3. 45 s heat shock at 42 °C
 4. 2 min incubation on ice
5. Add 250 (**500**) µl LB-medium and shake for 1 h at 37 °C 700 rpm
6. Spread cells on LB agar plates containing appropriate antibiotics

Formula :
$$V_{INSERT} = \frac{MW_{INSERT}}{MW_{VECTOR}} \times \frac{C_{VECTOR}}{C_{INSERT}} \times 3 \times V_{VECTOR}$$